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ANTIOXIDANTS IN FOOD AND PHARMACEUTICAL RESEARCH .

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Received: January , 2014; Accepted: February , 2014; Published on line: February , 2014

Abstract. In this work we provide a general overview about the natural and synthetic antioxidants and studies carried out to improve their potential. These compounds are able to scavenge free radicals that cause deterioration of food and pharmaceutical products during processing and storage. Today there are several known natural compounds with antioxidant properties that are extracted from plants, which are mainly phenols and polyphenols. The limit for the use of these compounds in the food and pharmaceutical industry as antioxidants concerns their poor solubility in hydrophobic environment. This limitation was overcame in the past years by the introduction of synthetic antioxidants, such as BHA (butylated hidroxy anisole), BHT (butylated hydroxyl toluene), TBHQ (tert butyl hydroquinone) and PG (propyl gallate). Unfortunately several authors showed that BHA, BHT, TBHQ and PG may present adverse effects on the health of living organisms. In recent years novel antioxidants have been synthesized from natural polyphenols in order to modify the hydrophilicity/lipophilicity balance and increase their biological functionalities arising from insertion of new functional groups or molecule moieties in pre-existing natural polyphenols. The aim of these modifications was to avoid the major adverse effects associated with the use of BHT, BHA, TBHQ and PG.

Key words. Natural antioxidants, oxidation, BHT, BHA, TBHQ, radicals, lipids , novel antioxidants.

Introduction. In the food industry an antioxidant is a substance having the technical function of delaying the oxidation of nutrients, such as lipids, sugars and proteins, whose oxidation leads to an inevitable deterioration of the organoleptic qualities of a food. Detrimental effects include undesirable formation of chemical compounds like aldehydes, ketones and organic acids that yield off-flavours [1,2]. Antioxidants must be inexpensive, nontoxic, effective at low concentrations (0.001–0.02%), capable of surviving processing (carry-through), stable in the finished products, and devoid of undesirable colour, flavour and odour effects [3]. Today in the food and pharmaceutical industry there is an extensive use of natural and synthetic antioxidants. Natural antioxidants such as polyphenols are primarily derived from plants, while the synthetic antioxidants are chemically produced. Common synthetic antioxidants used in the food industry are BHA, BHT, PG and TBHQ, that have been under scrutiny because of the potential injurious effects in the health field, (i.e. carcinogenic effects, DNA damage properties, allergic contact dermatitis and others adverse effects) [4-15]. Antioxidants can be classified as primary or long-term antioxidants and secondary or processing antioxidants. Primary antioxidants include hindered phenols and secondary aryl amines, while secondary antioxidants include organophosphites and thioesters. The first ones are active radical scavengers or hydrogen donors or chain reaction breakers while the secondary ones are peroxide scavengers [16]. Consequently, it is clear the importance of antioxidants in food conservation. Lipids and nutrients, contained in many kinds of foods such as chiken meat, red meat, diary food, seafood, can undergoe spoilage. In general there are three way of deterioration in foods; autolsis, microbiological spoilage, and lipid oxidation [17,18]. The oxidation of lipids by molecular oxygen (see Tab.1) generates free radicals and can be accelerated by light, heat, or metal ions. The peroxides formed during these reactions can, in turn, react with other lipids, fatty acids in particular, to form new kinds of peroxides. This second stage of lipid oxidation, referred to as the propagation phase, persists until the final termination stage, which occurs when two free radicals combine [19]. The importance of antioxidants in this context is to quench ROS and reactive radicals species in a termination reaction to prevent the degradation of foods. ***Natural antioxidant.*** Natural antioxidants are widely distributed in plants, animal tissues and microorganisms. These compounds protect the organisms from damage by oxidative stress caused by reactive oxygen species(ROS) and reactive nitrogen species (RNS), such as hydroxyl radical (•OH), hydrogen peroxide (H2O2), superoxide (O2 •-), nitric oxide (NO•), peroxynitrite (ONOO-), and others, that damage proteins, lipids, and DNA inside the cells [20]. The plant kingdom is a major source of natural antioxidants. In particular fruits, vegetables, spices, herbs, cereals, grains, oilseeds, leguminous seeds, teas, coffee and cocoa are the major sources of plant-derived antioxidants such as phenolics and carotenoids (see Tab. 2) [21]. Polyphenols are the most abundant natural antioxidants in nature. The term "polyphenols" refers to a class of natural compounds characterized by the presence of large multiples of phenol structural units [24]. This types of compounds are efficient antioxidants acting as free radical terminators or metal chelating agents [25]. The number and characteristics of these phenol structures underlie the unique physical, chemical, and biological properties of particular members of the class. They may be broadly classified as flavonoids (e.g. flavones, isoflavones, flavonols, flavonones, flavanols, anthocyanins, and condensed or hydrolysable tannins etc.) and non-flavonoids (e.g. phenolic acids, stilbenes and lignans) [25,26](Fig.1). Structurally most poly-phenols share the three-membered flavan ring system. Fig. 2 shows the ring labeling and numbering system of the flavan general structure, moreover there are also represented structures of catechol and gallol aromatic ring. In general typical flavan ring substituents are H, OH, OCH3, galloyl esters, protocatecuic esters or carbohydrate groups, depending on the compound [20]. Polyphenols are able to function as antioxidant in different ways; as hydrogen donor, as metal chelators and as protein inhibitors. In the first case the phenolic hydroxyl groups can reacts with ROS and reactive radical species in a termination reaction, which breaks the cycle of generation of new reactive radicals. The resultant radical form of antioxidant have a much greater chemical stability than the initial radical, due to an electron delocalization [27]. An example of an hydrogen donor and free radical scavenger mechanism is represented by the reaction between Kaempferol and DPPH (Fig.3). Kaempferol is a monohydroxy B-ring flavoinoid and DPPH is a common abbreviation for the organic compound 2,2-diphenyl-1-picrylhydrazyl, a stable free-radical molecule that is often used in a common antioxidant assay which provides an easy and rapid way to evaluate radical scavenging activity of specific compounds or extracts by spectrophoto-metry [28, 67]. After the donation of one hydrogen atom by Kaempferol to DPPH the resultant unpaired electron becomes highly delocalized and produces 10 resonance structures, a condition of high stability, infact the higher the number of resonance

Initiation reaction	$R^* + LH \rightarrow RH + L^*$	L^* = Lipid radicals
Propagation reactions	$L^* + O_2 \rightarrow LOO^*$	LOO^* = Lipid peroxyl radicals
	$LOO^* + LH \rightarrow LOOH + L^*$	LOOH = Lipid hydroperoxide
	$LOOH \rightarrow LOO^*, LO^*$, aldehydes	Breakdown of lipid hydroperoxide
Termination reactions	$2L^* \rightarrow L\text{-}L$	Dimers and stable products
	$2LO^* \rightarrow LOOL$	
	$2LOO^* \rightarrow LOOL + O_2$	

Table 1. Lipid peroxidation and radical / non-radical intermediates formation.

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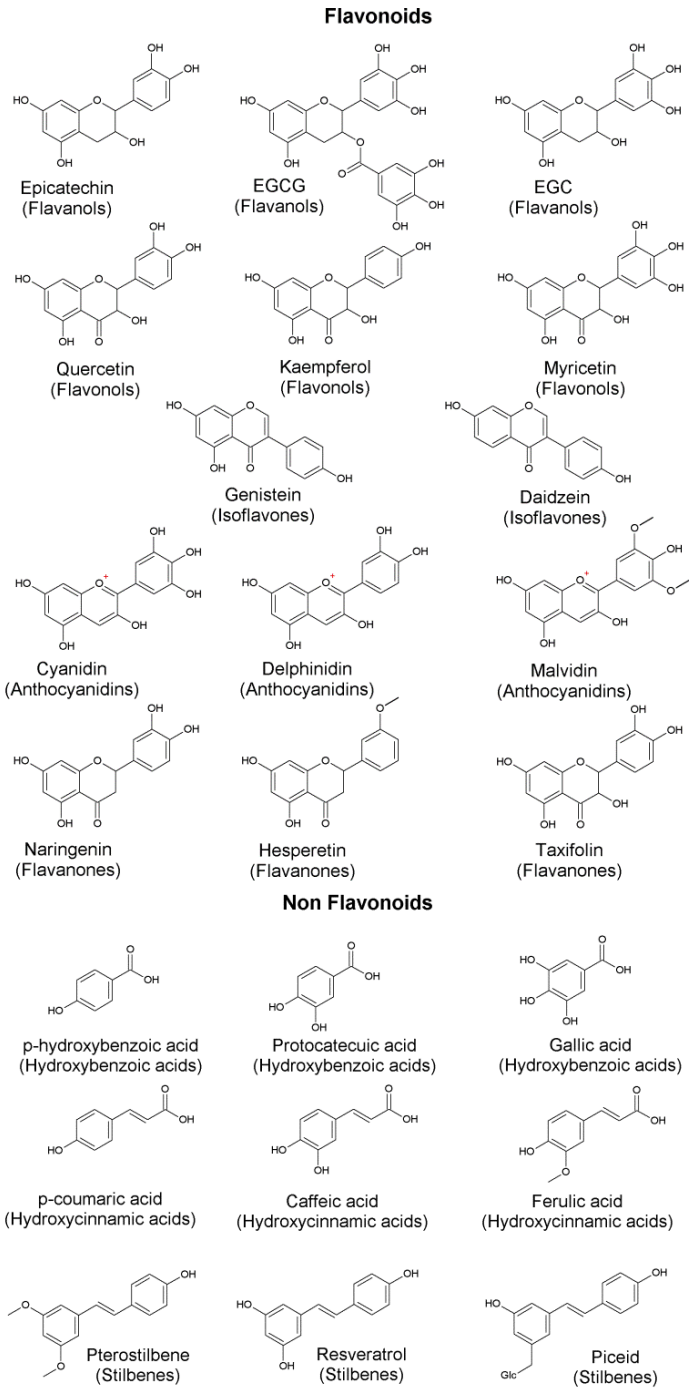


Figure 1. General classification of poliphenols in two groups Flavonoids and non Flavonoids. In brackets sub-groups.

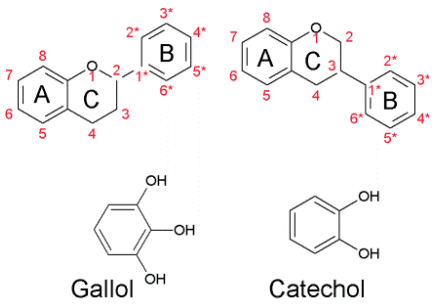


Figure 2. The ring labeling and numbering system of the flavan general structure.

structures, the lower the demanded energy for the formation of the free radical [29].The radical form of Kaempferol is able to donate another hydrogen atom to DPPH, forming the Kaempferol quinone. In the second case polyphenolic compounds are also able to form chelation complexes with metal ions involved in the production of free radicals, inhibiting this process [27]. For example Iron is implicated in many oxidative-stress-related pathways and conditions, and is the primary generator of H2O2 and Hydroxyl radical(•OH), the most reactive ROS known, that abstracts a hydrogen atom from biological substrates damaging biomolecules as lipids, proteins, DNA and other [20]. Iron, in nature, can be found as either ferrous or ferric ion with the latter form of ferric ion predominating in foods. Ferrous chelation may provide important antioxidative effects by retarding metal-catalyzed oxidation[30,31]. Acid phenolic hydrogens of poliphenols shows pKa values in the range of 7–9 [32], but in the presence of iron polyphenols are easily deprotonated at or below physiological pH and form very stable complexes. Precisely catechol and gallol moieties of poliphenols, when deprotonated, are able to chelate Fe2+ and Fe3+ ions to form complex with octahedral geometry [20]. However, since polyphenol compounds are so structurally varied and the complexes are pH dependent, they often exhibit variable coordination modes. At pH values previously reported polyphenols stabilize the Fe3+ relative to the Fe2+, moreover catecholate and gallate complexes of Fe2+ rapidly oxidize in the presence of O2 to give Fe3+-polyphenol complexes that cannot participate in the Fenton reaction[20,33].

Type of phenols	Examples	Food sources
Phenolic acids	Cinnamic acid	Cinnamon
	Vanilla beans	Vanilla bean
	Ellagic acid	Berries, walnuts
	Curcumin	Turmeric
	Rosmarinic acid	Rosemary
Isoflavones	Genistein	Soy bean and Fava bean
	Daidzein	
Flavones	Quercetina(flavonols)	Apple, kale, dill, ca-
	Kaempferol(flavonol)	persand, other fruits
	Taxifolin(flavanonols)	Citrus fruit
	Gingerol(flavanone)	Ginger
Flavanols	Catechin	Fruit skins e.g. grape, apple, berries or tea
	Epicatechin	
	Theaflavin	Tea leaves
Anthocyanidins	Anthocyanins	Berries, blackcurrant, bloodplum, cherry, redgrape, red apple, pomegranate, egg-plant, redcabbage/ radicchio, Spanish red onion, purple corn, purple carrot, black rice
Polyphenolic amides	Capsaicin	Chilli
	Avenanthramides	Oats
Other polyphenols	Resveratrol	Grapes, red wine, pistachio, peanut
	Lignans	Flaxseed, sesame seed, rye, wheat, oat, barley
	Cinnamaldehyde	Cinnamon
	Ellagitannins	walnuts

Table 2. Polyphenols subgroups and food sources [22,23]

Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH$

In the third case polyphenols may function as protein inhibitors. Precisely their structures formed by hydrophobic benzenoid rings and phenolic hydroxyl groups are capable to form hydrogen-bonds and have the potential to interact with proteins and inhibit enzymes involved in radical generation processes, such as various cytochrome P450 isoforms, lipoxy-

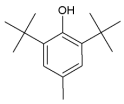
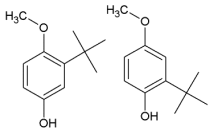
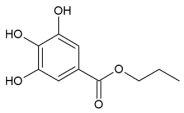
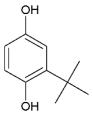
		BHT	BHA	PG	TBHQ
					
OTHER NAMES		2,6-bis(1,1-dimethylethyl)-4-methylphenol / 2,6-di-tertbutyl-p-cresol / 2,6-di-tertbutyl-4-methylphenol / Antracine 8 / Lonol CP / Dalpac / Impruvol / Vianol /Tenox / Tenox 8 / Sustane BHT.	Mixture of two isomers: 3-tertiary butyl-4-hydroxyanisole and 2-tertiary butyl-4-hydroxyanisole / (1,1-dimethylethyl)-4-methoxyphenol / Antracine 12 / Embanox / Nipantiox / Sustane BHA / Sustane 1-F / Tenox 4B / Tenox 5B.	n-propyl-3,4,5-trihydroxybenzoic acid / Gallic acid, propyl ester / Nipa 49 / Nipagallin P/ Tenox PG / Sustane PG.	2-(1,1-dimethylethyl)-1,4-benzenediol / mono-t-butyl hydroquinone / Sustane TBHQ / Tenox TBHQ.
FOOD USE		Breackfast cereals, Baked goods, potato chips, vegetable oils, snack foods, margarine, frozen seafoods, chewing gum base	Bakery products, meat product, spices, cereals, dehydrated mashed potatoes, beverage mixes, dessert mixes, nuts, vitamins, yeast, vegetable oils, animal fats, processed cheeses, margarine ,essential oils, chewing gum base.	Chewing gum base, non alcoholic beverages, margarine, mixed nuts, fresh or dry sausages, pre-grilled beef patties, rendered animal fat, pizza toppings and meatball.	Dry cereals, edible fats, margarine, pizza toppings, potato chips, poultry, dried meats, sausages, beef patties, vegetable oils.
"E" NUMBER		E321	E320	E310	E319
SYNERGISTS		BHA	BHT, propyl gallate, methionine, lecithin, thiodipropionic acid, citric acid, phosphoric acid.	BHA, BHT	BHA, citric acid
SOLUBILITY %	In water at 20°C	Insoluble	Insoluble	<1%	<1%
	In vegetable oils at 25° C	30% cottonseed, coconut, corn, peanut and soybean oils.	30% in cottonseed oil - 40% in coconut, corn, peanut oils -50% in soybean oils.	1% in cottonseed oil – 2% in soybean oils – Insoluble in con oil.	10% in corn, cottonseed and soybean oils…
	In ethanol solution 100% at 25°C	25%	>25%	>60%	25%
	In propylene glycol at20°C	Insoluble	70%	-	30%

Table 3. General Information about synthetic antioxidants.

genases, cyclooxygenase and Xanthine oxidase. [27,34] ***Synthetic antioxidant.*** Today among all synthetic compounds that have been evaluated for their efficacy as radical scavenger or for their other inhibitory effect, only four are widely used in foods, namely butylated hidroxy anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), and tert butyl hydro quinone (TBHQ) [35]. Introduction of alkyl groups into an aromatic ring is a useful and general approach to produce these antioxidants, in general alkylation involves attack of electrophilic carbon species on the ring. One of the well known reaction is the Friedel-Crafts alkylation, that uses an alkyl chloride as reagent and anhydrous aluminum chloride as catalyst [36]. The choice of reagents and conditions depends upon the alkyl group and the reactivity of the ring. Industrially BHT is prepared by the reaction of p-cresol (4-methylphenol) with isobutylene (2-methylpropene) catalyzed by sulfuric acid while BHA is prepared from 4-methoxyphenol and isobutylene in a similar reaction [37]. BHT is a white crystalline solid with a faint characteristic odor. It is insoluble in water and in propylene glycol, but is freely soluble in alcohol. It is used as a chemical antioxidant for food, cosmetics, and pharmaceuticals much like butylated hydroxyanisole (BHA). On the other hand BHA is a mixture of 3-tert-butyl-4-hydroxyanisole (typically 90% w/w) and 2-tert-butyl-4-hydroxyanisole. BHA is insoluble in water, but is freely soluble in alcohol and in propylene glycol. These phenol derivatives react with the free radicals slowing the rate of lipids autoxidation that can lead to changes in the food's color and taste. BHT and BHA are also used as antioxidants in plastics, elastomers and petroleum (lubes, greases and waxes), practically bigger market size than food field. TBHQ (2-tert-butylhydroquinone) is a white, crystalline solid insoluble in water but soluble in alcohol and in ether. It can be synthesized selectively from hydroquinone (HQ) and isobutylene by alkylation in the presence of H_3PO_4 or H_2SO_4/H_3PO_4 catalyst [38] or by alkylation with tert-butanol. In foods, it is used as a preservative for unsaturated vegetable oils and many edible animal fats [39]. It's also used in formulating varnish, lacquer, resins and oil field additives. It is used as a fixative in perfumery to reduce the evaporation rate and improve stability and as a stabilizer to inhibit the autopolymerization of organic peroxides. PG (propyl gallate or propyl-3,4,5-trihydroxy benzoate) is a white, odorless powder having a slightly bitter taste. It is slightly soluble in water and freely soluble in alcohol and in ether. This compound is an ester formed by the condensation of gallic acid and propanol. Since 1948 it is used in food industry as antioxidant to prevent oxidation of foods containing oils and fats [40]. In addition to the food industry, the

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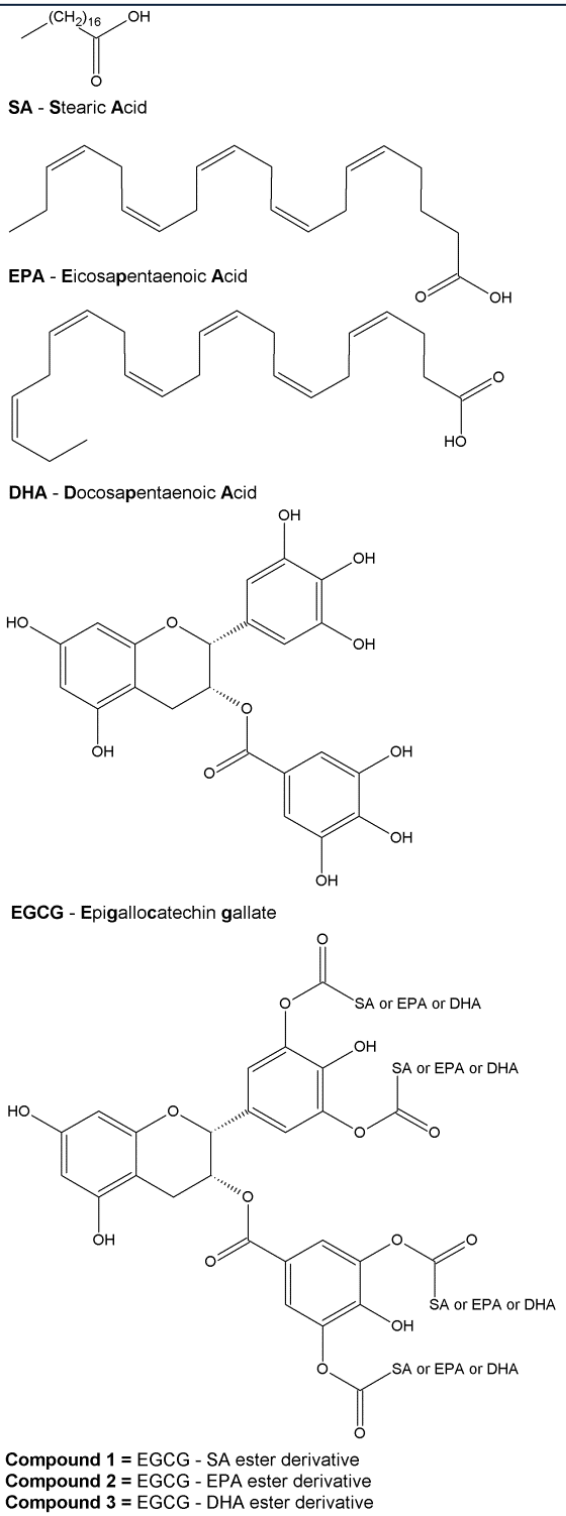


Figure 4. stearic ester, eicosapentaenoic ester and docosaesanoic ester derivatives of EGCG.

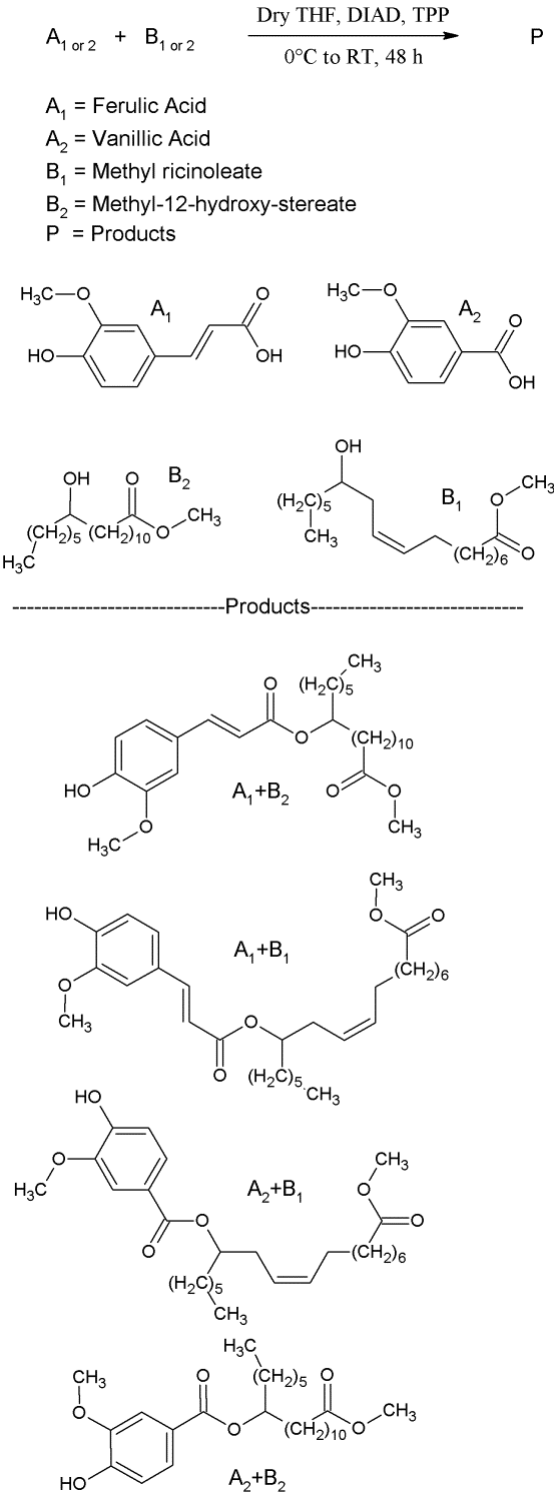


Figure 5. Esterification reaction of ferulic acid and vanilic acid with methyl ricinoleate and methyl-12-hydroxystereate.

PG is used as a anti-fade reagent in fluorescence microscopy to reduce photo-bleaching of fluorescences such as rhodamine and fluorescein [41] and is also used as antioxidant in cosmetics, hair products, adhesives, and lubricants. Tab. 3 shows all the most important information about the antioxidants mentioned above, along with their respective E numbers, that identify substances which can be used as food additives within the European Union and Switzerland (the "E" stands for "Europe"). They are commonly found on food labels throughout the European Union. The additives for food use undergo to a process of safety assessment before being approved by European and international countries. In Europe, the evaluation is carried out by the Food Safety Authority (EFSA), and at international level by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). As previously mentioned, these compounds in food have the technical function of antioxidant preservatives. So they prevent oxidative rancidity development in oil-containing foods by terminating free radicals formed during autoxidation of unsaturated lipids. Moreover they possess antimicrobial activity as a phenolic compound [35].

Novel antioxidant. Common synthetic antioxidants used in the food industry, namely BHA, BHT, PG and TBHQ offer good protection during storage, but have been under scrutiny owing to the growing concern over their potential carcinogenic effects [4-15]. Moreover natural polyphenolic compounds are water-soluble due to the hydrophilicity arising from their hydroxyl groups. This may compromise their effective application as antioxidant in lipophilic systems, such as fats, oils, lipid-based food or cosmetic formulas and emulsions, as well as biological environments [21]. As a consequence, the development of compounds with better antioxidant capacity and less toxicity is desirable in health (es. prevention of deseases), in food, in pharmaceutical and cosmetic field to improve shelf life of respective consumer products [42]. A possible way to prepare novel anti-

oxidant from natural phenols and polyphenols is to manipulate lipophilicity/hydrophilicity balance (HLB) through chemical structure modification of these compounds [21]. The change in the solubility characteristics of natural antioxidants, without changing the molecular moieties responsible for antioxidant activity, can enhance antioxidant application in food, pharmaceutical and cosmetic fields [42]. In general the synthesis of esters or amides derivatives of polyphenols with fatty acids or fatty alcohols represent a general way to make novel antioxidants. Moreover the conjugation between polyphenols with fatty acids may provide additional advantages by introducing new bioactive functional groups to the antioxidant molecules[21]. The chemical literature is full of examples of esterification of phenolic acids with aliphatic alcohols or poliphenols with fatty acids. Specifically these last were used to synthesize ester derivatives of ascorbic acid, tyrosol, rutin, protocatechuyl alcohol, vanillyl alcohol, esculin, naringin, genistein and daidzein[43-48], while aliphatic alcohols have been used to esterify ferulic, chlorogenic, cinnamic, sinapic, p-coumaric and caffeic acids [49-53]. In the following paragraphs some examples will be reported. ***Fatty acids-Epigallocatechin gallate (ECGC) conjugation.*** Green tea is an important source of epigallocatechin gallate (EGCG), a natural powerful antioxidant that is effective in radical scavenging and metal chelation [54-55]. EGCG is the predominant one among the catechins in tea, a group of flavonoids that are also responsible for the characteristic colour, flavor and aroma of this famous drink. Like many other polyphenols, EGCG is poor soluble in lipophilic systems and this fact can greatly restrict its use as antioxidant in fats, oils, lipid-based food or cosmetic formulas and emulsions, as well as biological environments [56]. In recent works [57-58] epigallocatechin gallate was esterified by acylation with acyl chlorides of selected long-chain saturated or polyunsaturated fatty acids such as SA (stearic acid), EPA (Eicosapentaenoic acid) and DHA (docosa-esanoic acid) to produce three esters (Fig. 4) with enhanced lipophilicity [56]. These products showed higher radical scavenging activity against peroxyl radicals than the parent EGCG molecule in acetone/water solvent medium(for more information see [57]) and better or comparable antioxidant activities to that of the parent EGCG in biological model systems including LDL-cholesterol, DNA and liposome[56]. Moreover EGCG derivatives have demonstrated the ability to chelate prooxidant metal ions Fe2+ and showed excellent antiviral activities in inhibiting HCV protease and α-glucosidase, which were not significant for EGCG [57]. In conclusion these lipophilic derivatives of EGCG could be considered for use in food preservation and health promotion. ***Ricinoleate-phenolic acids conjugation.*** K.K. Reddy et al. published a very interesting work in 2012 [42] focused on the synthesis, characterization and evaluation of four novel antioxidants, synthesized by conjugation between the phenolic acids as ferulic and vanillic acids with methyl ricinoleate and methyl-12 -hydroxy stereate (see Fig.5), the first is the principal unsaturated fatty acid contained in castor oil, the last one is itssaturated analogue. Precisely about 90% of the fatty acid content in castor oil is the triglyceride formed from ricinoleic acid, that is known to exert laxative, analgesic and anti-inflammatory effects in human health [59] . The choice of such compounds has allowed the authors to assess the influence of unsaturation in the alkyl chain and in the side chain of phenolic acid on the antioxidant activity. The reactions to prepare four novel antioxidants were performed according to the chemoselective Mitsunobu esterification method [60]. Moreover antioxidant, antibacterial and antifungal activity of these novel molecules were evaluated. The radical-scavenging activity of the synthesized compounds was determined through the use of DPPH radical scavenging assay in a polar homogeneous medium. In this polar conditions the four novel molecules, after lipophilisation, showed decreased radical-scavenging activity respect to the starting free phenolic acids. The authors explained this fact considering the increased hydrophobicity of the synthesized phenolipids. It is also to be noted that the ferulic acid derivatives showed better radical-scavenging activity than the vanillic acid derivatives . This fact proves the importance of the allylic double bond of ferulic acid in stabilising the phenoxy radical. Moreover the phenolipid products have demonstrated to inhibit the autoxidation of linoleic acid in Tween 20 micellar system (Tween represent a common commercial brand for nonionic surfactants polysorbates) significantly more than ferulic and vanillic acids and similar to DGG (dodecyl gallate), the well known grade antioxidant. This fact is particularly important because antioxidants are widely used in cosmetic and food products to inhibit lipid peroxidation and most of these products exist in a formulated complex emulsion system[42]. Moreover the prepared compounds showed interesting antifungal properties but none of the synthesized phenolic

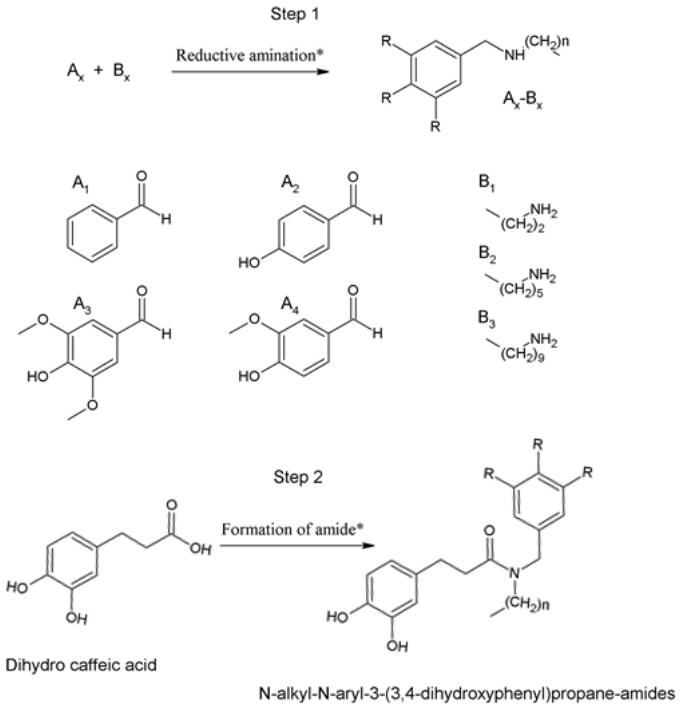
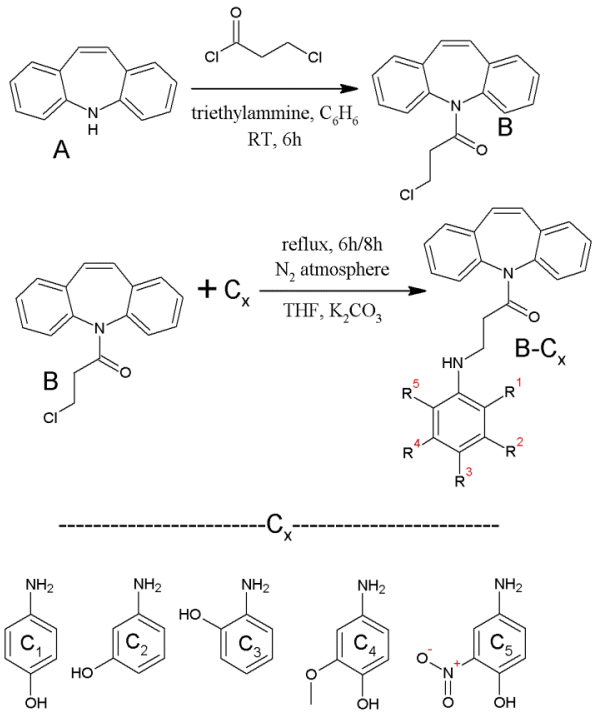


Figure 6. Two step sinthesys to prepare N-alkyl-N-aryl-3-(3,4-dihydroxy phenyl) propane-amides.



A = 5H-dibenz[b,f]azepine
B = 3-chloro-1-(5H-dibenz[b,f]azepine-5-yl)propan-1-one
C_1 = 4-aminophenol
C_2 = 3-aminophenol
C_3 = 2-aminophenol
C_4 = 4-amino-2-methoxyphenol
C_5 = 4-amino-2-nitrophenol

Figure 7. Reaction scheme for the synthesis of substituted amino phenol analogues of tricyclic amine 5H-dibenz[b,f]azepine.

lipoconjugates showed any activity against the studied bacteria (for more information see [42] and references there cited). In conclusion these novel compounds have shown to be potential and interesting antioxidant additives for the storage of lipids against oxidative stresses. ***Dihydro-***

caffeic acid amide derivatives as novel antioxidants. In 2012 was published a work [61] based on the synthesis of a variety of new N-alkyl-N-aryl-3-(3,4-dihydroxyphenyl) propane-amides (Fig.6) as novel caffeic acid amide antioxidants. These compounds contain the aromatic structure of the dihydro-caffeic acid, that results to have higher radical scavenging and antioxidant activity compared to caffeic acid [61] . The choice to synthesize amide derivatives of caffeic acid is based on the fact that many reports have demonstrated the potential of these derivatives in the food and health fields. For example in 2001 Rajan P. et al. reported a new caffeic acid amides with good antioxidant activity and enhanced metabolic stability [62]. Furthermore in 2002 was published a work in which the authors described the synthesis of 4-hydroxyphenylacetic acid amide, which possess potent analgesic activity [63]. Moreover the synthesis of caffeic acid amides, possessing antimicrobial activities, was reported in 2010 [64]. As is shown schematically in Fig.6 a variety of new N-alkyl-N-aryl-3-(3,4-dihydroxy phenyl) propane-amides were prepared in two steps synthesis. In the first step amines Ax-Bx were obtained by reductive amination between four different aldehydes that are Benzaldehyde(A1), 4-hydroxybenzaldehyde(A2), vanillin(A4), syring aldehyde(A3) and three different amines (propylamine, hexylamine decylamine) for wich the imines produced were subsequently reduced with sodium borohydride. In the second step antioxidants dihydro-caffeic acid amide derivatives were prepared through coupling reaction between dihydro-caffeic acid (DCA) and the corresponding amines Ax-Bx, using BPO as a coupling reagent. DPPH assays demonstrated a significantly higher radical scavenging activities of novel antioxidants N-alkyl-N-aryl-3-(3,4-dihydroxy phenyl) propane-amides than a-tocopherol, BHT and DCA. Furthermore, when compared to α-tocopherol and BHT, the new compounds offered better protection to polyunsaturated oils both under storage and frying conditions [61]. ***Novel 5H-dibenz[b,f]azepine derivatives.*** Comparing to the importance of the O-H groups in phenols, that give good antioxidant capacity against radicals as idrogen donors, also the aryl secondary amines are able to act as chain transfers and chain terminators against free reactive radicals [64,65]. Recently H. Vijay Kumar and Nagaraja Naik published a work in which the tricyclic amine 5H-dibenz[b,f]azepine (A, Fig.7), that has proven to possess along with some of its derivatives antiallergic activity, specifically antihistaminic activity, spansmolytic, serotonin antagonistic, anti- convulsive, antiemetic, antiepileptic, antiinflammatory, sedative and fungicidal action[66], was used as the starting compound for preparing its substituted aminophenol analogues [67]. Fig.7 showed the synthesis scheme. The first reaction reports the N-acylation of 5H-dibenz[b,f] azepine(A) with 3-chloro propionyl chloride, while the second reaction represents a base condensation between different aminophenols and substituted aminophenols with 3-chloro-1-(5H-dibenz[b,f] azepine-5yl)propan-1-one(B), that is the product of the first reaction. The radical scavenging activity (RSA) was determined for all the synthsized compounds through the DPPH radical scavenging assay. Moreover the antioxidant activity in inhibition of lipid peroxidation was also evaluated, using β-carotene-linoleic acid assay [67], and inhibition of the oxidation of poly unsaturated fatty acid (PUFA) of human Low-density lipoprotein (LDL). LDL is one of the five major groups of lipoproteins, that are aggregates containing both proteins and lipids and enable the transportation of fats such as cholesterol, phospholipids, and triglycerides, within blood stream. Oxidation modification in LDL may play an important role in the pathogenesis of atherosclerosis [68] and the protection of LDL by dietary antioxidants may therefore reduce atherogenesis [69]. 3-chloro-1-(5H-dibenz[b,f]azepine-5yl)propan-1-one (B Fig.7) showed no significant radical scavenging activity over DPPH, while after its condensation with compounds Cx (Fig.7) to give 5H-dibenz[b,f] azepine analogues (B-Cx, Fig.7) containing aminophenols and substituted aminophenol groups showed comparable or slightly less radical scavenging activity against DPPH than the ascorbic acid and BHA used as reference. Regarding to LDL oxidation (for more detailed information see [67]) compound (B) showed less activity with respect to compounds B-Cx in inhibiting LDL oxidation. Among the six compounds synthesized and evaluated with β-carotene-linoleic acid assay [67] compound B showed less activity, while compounds B-C4 and B-C5 showed excellent activity in inhibiting lipid peroxidation followed by aminophenol analogues B-C1, B-C2 and B-C3. In conclusion H. Vijay Kumar and Nagaraja Naik have demonstrated that the compounds B -Cx have significant activity as antioxidants in different in vitro model systems. These effects as antioxidants may be useful in health and food fields in which free radical oxidation plays a fundamental role.

Conclusions. Antioxidants are very important compounds for the correct conservation of food and pharmaceutical products. Nowadays there is an extensive use of synthetic antioxidants such as BHT, BHA, TBHQ and PG, which may present several health related problems. Future developments are focused mainly on the use of natural antioxidants with specific biological and chemical charachteristics, in order to improve the conservation of food and pharmaceutical products and retard the detrimental effects of organoleptic proprieties. Moreover different research groups are working on the development of modified antioxidant natural compounds, to improve some chemical characteristics of such molecules i.e. liposolubility. These efforts demonstrate that the field of antioxidants research will remain quite active in the future.

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